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CONCENTRATION ET PROPRIÉTÉS DES ANTICORPS ANTI-ACIDE RIBONUCLÉIQUE

CONCENTRATION AND PROPERTIES OF ANTI-RIBONUCLEIC ACID ANTIBODIES

by

Emanoil Barbu and Jacques Panijel

Summary

It is possible to concentrate and purify anti-RNA antibodies present in anti-ribosomal serum. This purification employs precipitation with RNA. These antibodies will also precipitate with certain synthetic polyribonucleotides.

We have previously demonstrated[1,2] that antisera against bacterial ribosomes will precipitate not only with the ribosomes used for immunization but also with ribosomes from various other sources. Certain of these antisera will precipitate even with purified ribonucleic acid (RNA)[3]. Described in this note is a method for the concentration of anti-RNA antibodies starting with anti-ribosomal sera which are incapable of precipitating spontaneously with free RNA. This method is based on the hypothesis that RNA represents the fundamental common antigen among ribosomes of all sources and that it is the only common antigen among ribosomes originally used for preparation of the immune serum and the so-called 'distant' heterologous ribosomes with which a number of the precipitating antibodies in sera react only feebly[4]. Consequently, the 'distant' heterologous ribosomes ought especially to carry down in the specific precipitate the anti-RNA antibodies which having been concentrated in this manner, are further purified.

The method of preparation used is shown as follows:

(A) To 120 ml of goat antiserum for E. coli K_{12} 3000 ribosomes, are added 12 mg of Fusiformis fusiformis ribosomes. After sitting for 16 hr at 4°C, the precipitate is collected, which, after washing, contains 45 mg of protein and 7 mg of RNA. This precipitate is suspended in 10 ml of buffer 'A' (0.14 M NaCl in 0.005 M tris(hydroxymethyl) aminomethane buffer, pH 7.5); Setting for 24 hr at 37°C activates the latent ribosomal RNase so that it hydrolyzes the RNA in the precipitated ribosomes; this follows the dissociation of the ribosomes themselves and, consequently, those of the specific precipitate. The insoluble protein of the ribosomes and possibly the antibodies that are bound to them, remain as a precipitate whereas the products of RNA hydrolysis and the anti-RNA antibodies are dissolved; after centrifugation, one recovers in the supernatant 80 %, occasionly more, of the starting protein. The immune globulins are then precipitated with 33 % saturated ammonium sulfate and then redissolved in buffer 'A'. After dialysis against buffer, 34.5 mg of antibody is recovered in the supernatant.

(B) The antibody suspension is reacted with 500 µg of RNA. This was prepared from Ehrlich ascites cells by six successive phenol extractions and reprecipitated with 1.0 M NaCl.

The anti-RNA reaction allows one to obtain a specific precipitate containing 4.5 mg protein and 175 µg RNA. The percentages of RNA and protein precipitated are 35 and 13 % respectively. This antibody/antigen ratio is equivalent to 26.

The following table shows the results obtained with other preparations concentrated under similar conditions:

origin and means of precipitation of γ-globulin	γ-globulin in mixture	RNA in mixture	γ-globulin in precipitate	RNA in precip.	antibody antigen
	µg	µg	%	%	
goat antiserum for *E. coli* K_{12} 3000, precipitated with *Streptococcus* A_{23} ribosomes	1400	25	12	24	28
	1400	50	17	14	34
	1400	100	26	12	30
goat antiserum for *E. coli* K_{12} 3000 ribosomes, precipitated with *Fusiformis fusiformis* ribosomes	420	65	24	9	17
	840	65	20	15	17
rabbit antiserum for *Proteus vulgaris* ribosomes, precipitated with *Streptococcus* A_{23} ribosomes.	1500	40	20	85	9
	1500	120	32	46	9
	1500	240	22	18	8

(C) The purification of anti-RNA antibodies is affected by the following: the specific precipitate (antibody+RNA) obtained in (B) is redissolved in buffer 'A' and treated with RNase (5µg/ml) for 24 hr at 37°C. The antibodies which go into solution are precipitated twice with 33 % saturated ammonium sulfate (this eliminates significant traces of RNase) and then dialyzed against buffer 'A'. This preparation, which contains 3.5 mg of antibody, gives with 150 µg of ascites RNA, a specific precipitate which is treated as before. At the end, one obtained 2.35 mg of purified antibody.

Eventhough the final yield is relatively poor, the precipitating ability of these antibodies has been notably improved. In fact, with 1 mg of antibody and 50 µg of RNA mixed in buffer with 0.01 M $MgSO_4$ added[6] one obtains a precipitate containing 318 µg of protein and 35 µg of RNA which are 32 and 70 % respectively of the starting quantities. The antibody/antigen ratio is 9.

The study of the nature and properties of anti-RNA antibodies permit one to now speculate on a number of points:

First- the anti-RNA antibodies are entirely γ-globulin. One of the preparations (rabbit serum no. 92 against *E. coli* K_{12} 3000 ribosomes, precipitated with *Alcaligenes fecalis* ribosomes) when subjected to immunoelectrophoresis gave only a single band with goat antiserum against rabbit whole serum; this band corresponded to the one for γ-globulin in whole rabbit serum. When ribosomes were used in the immunoelectrophoresis experiment, again only one band corresponding to γ-globulin was obtained.

Second- it has been demonstrated that anti-RNA antibodies no longer precipitate RNA after it is treated with RNase[3]. These antibodies are also capable of precipitating with polymerized RNA from diverse sources and will also precipitate with synthetic polynucleotides[7]. This is true moreover not only of antisera that precipitate strongly with RNA but also of the purified and concentrated antibodies. In the case of rabbit antiserum no. 55 against E. coli B ribosomes, which precipitates spontaneously with RNA, 40 to 70 % of the polyadenylic acid added is precipitated. In a similar manner, about 46 % of the polyadenylic acid was precipitated with purified antibody from a goat antiserum against E. coli K_{12} 3000 ribosomes (precipitated with ribosomes from Streptococcus A_{23}).

Third- It has not been possible, because of the concentrations employed, to recover in a specific precipitate either all of the antigen or all of the antibody. This may be due to the presence of soluble complexes or an inhibitor effect of an RNA analog as is evidenced in the precipitation of heterologous ribosomes[3].

These results confirm the existence of anti-RNA antibodies. But one can still question whether RNA represents in itself the antigenic moiety or whether it only constitutes a portion of a more complex antigenic moiety. Some of our experiments appear to give some credit to the latter hypothesis.

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(3) E. Barbu and J. Panijel, Comptes rendus, 250, 1960, p. 1382.

(4) E. Barbu, J. Panijel, and G. Quabh, Ann. Inst. Pasteur, 100, 1961, p. 725.

(5) W. Moeller and B. Bödtker, Fed. Proc., 20, 1961, p. 357.

(6) According to Moeller and Bödtker[5] the degradation of RNA by γ-gloublins, cited by Brown, Ellem, and Colter, can be inhibited by magnesium.

(7) This was furnished to us by Mme. Grunberg-Manago.

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